

SPECTRAL CHANGES OF LUNG CANCER SERUM IN THE PROCESS OF TUMOR EVOLUTION

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Abstract- The discrimination and changes of serum fluorescence and Raman spectrum for normal human, lung cancer patient and one after operation in different period and extent were studied. We kept close watch on the tumor progression of a group of patients, and measured their serum spectra using 488.0nm and 514.5nm excitation of an Ar-ion laser once a week. There was no apparent change observed in fluorescence spectrum in different period. However, the relative intensity of three Raman peaks (mode A, B and C) decreased every week later. For quantitative analysis of such changes, a parameter I_r (relative intensity of C Raman peak) was introduced and I_r -value was calculated. Calculation showed that I_r -value was degressive with tumor evolution, but \hat{a} (I_{r5145} / I_{r4880}) varied irregularly. To the end, no Raman peak was observed. We assumed that three Raman peaks were derived from beta carotene. It indicated that the content of beta carotene decreased with the aggravation of lung cancer.

Keywords: lung cancer, serum, spectrum, diagnosis

I. INTRODUCTION

Over the past ten years, the medical community to characterize various physicochemical properties of biomolecular has attracted fluorescence spectroscopy. Fluorescent dyes used in complex biomolecular systems have enabled the researchers to obtain information about conformational changes in muscles and nerves, polarity of the surrounding environment, dynamical conformation of molecules in membranes and the secondary structure of DNA and RNA [1]. Hematoporphyrin derivatives (HPD) and other fluorescence materials such as hematoporphyrin monomethyl ether (HMME) are currently being employed as a fluorescent marker for cancer detection. However, these extrinsic fluorophores may alter the native

environment of cell and tissues [2].

Recently, the photo-physical properties of native fluorophores of cells, tissues and their structures have been considered as an useful parameter to study alterations in the functional, morphological and microenvironmental changes in the cells and tissues [3~6]. And the native fluorescence spectrum of serum has been researched for the purpose of differentiating cancerous from normal cases [8~9].

In our former researches, spectral methods of laser-induced fluorescence spectroscopy (LIF) for diagnosis of cancer have been investigated. We have used $\hat{a} < 1.0$ as a criterion for the diagnosis of lung cancer, and obtained an accuracy of 77.36%. \hat{a} is ratio between the relative intensity of Raman peak using 514.5nm and 488.0nm excitation. However, the correlation between \hat{a} and tumor evolution was not aware of.

In lung cancer and other cancer diseases some chemical compositions in serum may change dramatically as disease progress. And biochemical changes associated with disease provide important clues for diagnosis. Therefore it is essential to find such changes and

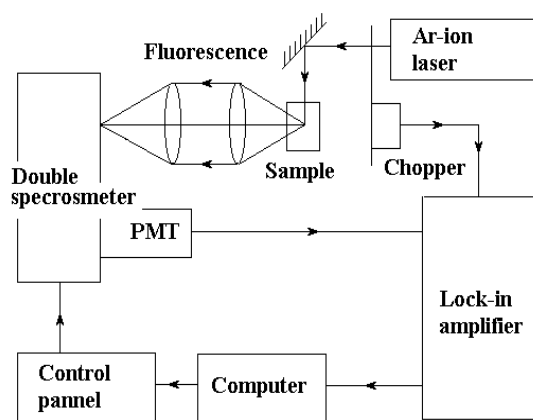


Fig. 1: instrument

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determine the connection between them and cancer evolution for diagnosis of cancer using LIF. In this paper, we measured serum spectra of a group of lung cancer patients once a week, and investigated intensity change of Raman peaks in such spectra. In addition, I_r -value in each spectrum was calculated. We compared I_r -value and \hat{a} -value obtained from the same person's spectra in an attempt to find some valuable results about I_r and \hat{a} in the process of tumor growth.

II. METHODS AND MATERIALS

Twenty-six subjects were entered in this study, but seven exited during the course of experiment. All samples were obtained from Tumor Hospital of Liaoning Province and were exactly diagnosed as terminal stages of lung cancer.

Spectra were collected with a double spectrometer (it can be precisely controlled by computer) equipped with a PMT. After amplified by a lock-in amplifier, spectral data were input into computer and transacted. The spectral range scanned was from 520nm to 640nm or from 500nm to

620nm at a spectral resolution of 2 cm^{-1} . And the frequency of chopper was 700HZ. Fig.1 shows the main parts of our instrument: Ar-ion laser (made in 772 factory

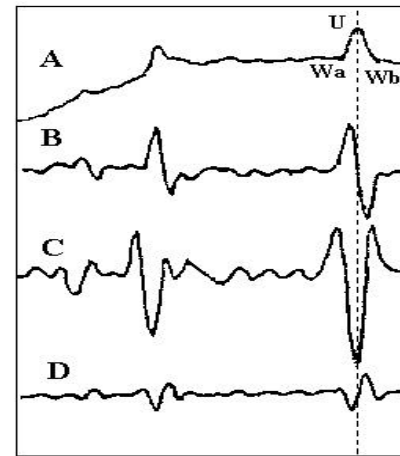


Fig.2 several types of spectra calculated during the course of data processing (A: original spectrum; B: first derivative spectrum; C: second order derivative spectrum; D: third order derivative spectrum)

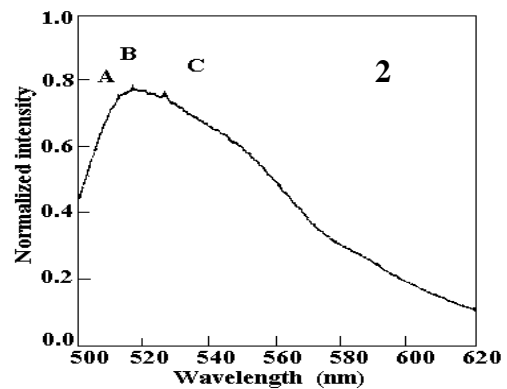
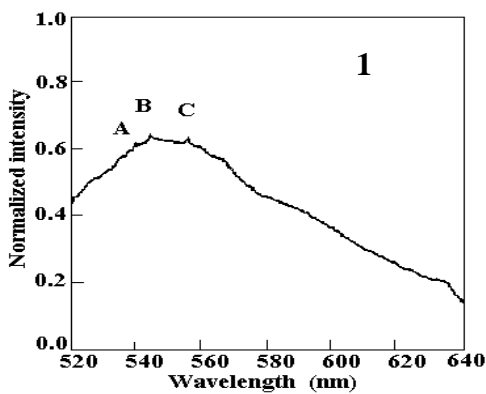


Fig. 3 Normalized spectrum in the first week (1. 514.5nm excitation, 2. 488.0nm excitation)

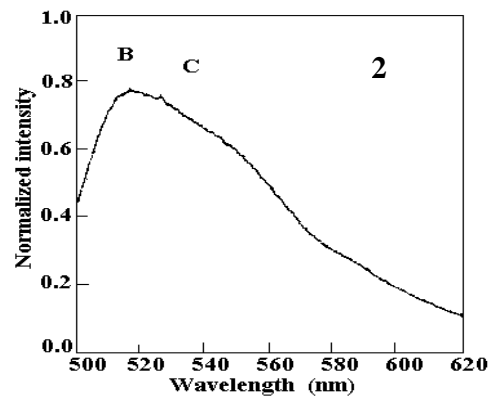
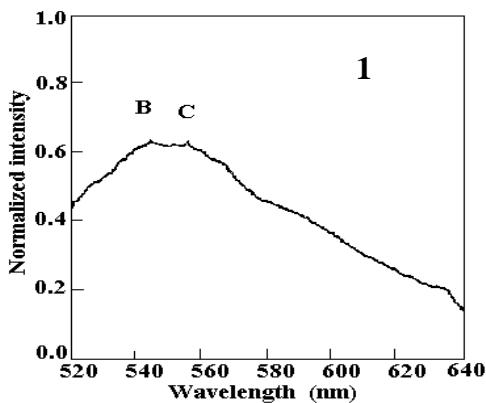


Fig.4.. Normalized spectrum in the third week (1. 514.5nm excitation, 2. 488.0nm excitation)

in nanjing), PMT (R456 model), Lock-in amplifier (391A model), double spectrometer (HRD-1 model). The wavelengths of 488.0nm and 514.5nm were chosen for excitation.

We collected samples once a week, and obtained eight sets of specimens in different period for each subject (but several not). And for each sample, two spectra were measured: (1) the spectrum from 520nm to 640nm excited by 514.5nm; (2) the spectrum from 500nm to 620nm excited by 488.0nm. What we recorded was relative intensity (absolute intensity divided by maximum intensity) in order to reduce such interference as the undulation of laser power. And for the purpose of lessening influence of other harmful factors, we sampled several data at each wavelength, and then recorded the average value. And in the process of original data transaction, method of least squares was used to smooth spectra. Due to the resolution improvement of second order derivative spectrum compared with the original, it was utilized to find the position U of Raman peak (mode C) and two inflexions (W_a and W_b) nearest (see Fig.2). Supposing that fluorescence intensity is linear with wavelength between inflexion W_a and W_b (it is acceptable because

the value of second order derivative spectrum of fluorescence is very small), we got fluorescence intensity at location U and then I_r .

III. RESULTS AND DISCUSSION

Of twenty-six subjects, seven exited during the course of our experiment for different reasons. As a result, only nineteen patients' spectra were recorded. Typical sets of spectra from the same person serum are presented in Fig.3, Fig.4 and Fig.5 (in the first, third and fifth week respectively). In Fig.3, three Raman peaks (mode A, B and C, mode C is the strongest one) can be observed, but not very distinct due to the strong interference background. However, there is no peak A in Fig.4, and intensity of peak B and C decreases compared with Fig.3, but fluorescence spectrum almost maintains its former shape. In Fig.5, we are not capable of observing any peak directly, but can find peak C by means of derivative of spectrum (only in spectrum excited by 488.0nm, not by 514.5nm). In the sixth week and later, even mode C cannot be detected.

There are some differences in fluorescence spectra in different period, such as intensity, peak position. Those can be attribute to some biochemical changes occurring in serum as disease progress. For further quantitative

TABLE I_r-value and \hat{a} -value in different period

Secon d week		First week	Third week	Fourth week	Fifth week
1.98%	I_{r4880}	2.45%	1.44%	0.92%	0.51%
1.71%	I_{r5145}	2.31%	1.37%	0.86%	
0.864	\hat{a}	0.943	0.951	0.935	

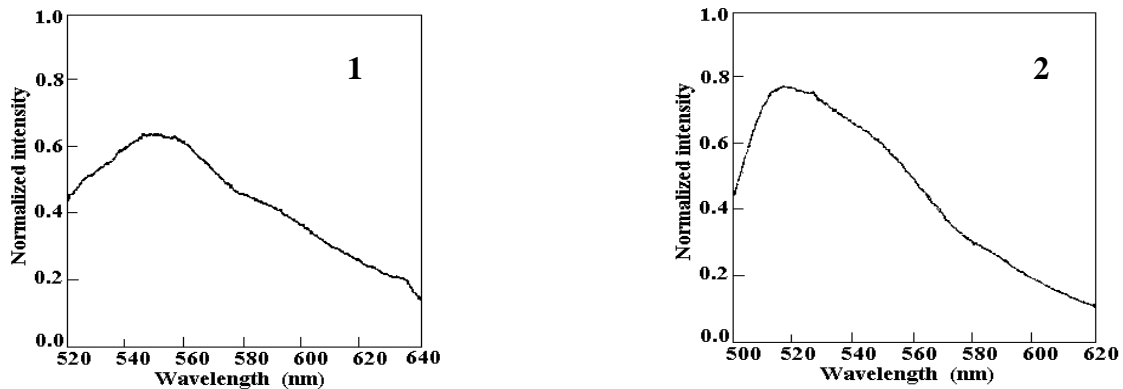


Fig.5. Normalized spectrum in the fifth week (1. 514.5nm excitation, 2. 488.0nm excitation)

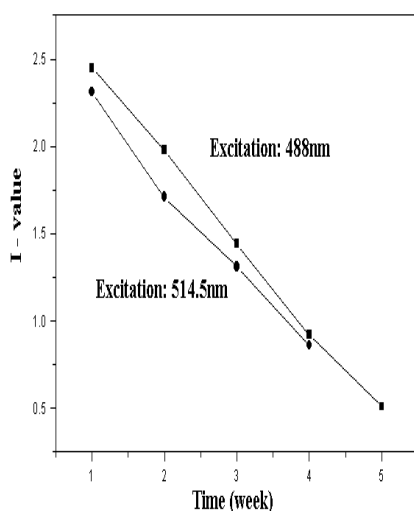


Fig.6: Connection between I_r -value and tumor evolution (in different period)

study, we calculated I_r -value and \hat{a} -value of such spectra (see table 1), and made a graph of I_r -value and time (see Fig.6). It demonstrates that I_r is approximately linear with time in this experiment. And for \hat{a} , it is impossible for us to draw some conclusions because it varies irregularly. But it should be pointed out that \hat{a} -value is less than 1.0 in all spectra that we measured.

In serum spectrum, three Raman peaks are almost the same as resonance Raman spectrum of beta carotene in carbon tetrachloride solution, whether peak location or intensity distribution^[10]. We assumed that they were derived from beta carotene emission. Beta carotene is a kind of carotenoids and can be translated into vitamin A. In epidemiology, studies showed that the incidence of cancer and the content of beta carotene are closely relevant. The higher the content of beta carotene, the less incidence of cancer^[11]. In 1996, Ma qingyun, by means of chromatogram, found that the content of beta carotene in stomach cancer patient's serum (0.3-0.9 μ mol/l) was obviously lower than normal person's one (1.5-3.2 μ mol/l)^[13]. And some researches demonstrated that beta carotene, to some extent, can restrain the growth and progression of tumor cells^[12]. Our result indicated that the content of beta carotene decreased with aggravation of lung cancer. It means

that beta carotene has close relationship with cancer. Such conclusion agrees well with former studies in chromatogram and epidemiology.

Due to the endogenous fluorescence background presented in blood plasma, which is of the order of a million times more intense, the relatively weak Raman signals are difficult to extract. Therefore, near-IR radiation may be used for further studies.

IV. CONCLUSION

The content changes of chemical components in serum are a major factor that results in serum spectral changes. In this paper, we studied spectral changes of lung cancer patients in different period, found the intensity of three Raman peaks in spectrum decreased as disease progress. Based on the supposition that three Raman peaks are derived from beta carotene in serum, a conclusion was made that the content of beta carotene decreased as disease progress. It may be useful in cancer diagnosis.

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